EFFECTS OF VARIOUS TENDERIZATION STRATEGIES ON NON-FED BEEF PALATABILITY AND TENDERNESS

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ABSTRACT

Effects of exogenous enzyme combinations and a mechanical tenderization method were evaluated by myofibrillar fragmentation (MFI), sensorial attributes and oxidative stability (TBA) of the deep pectoral (PEC: n=120) and *biceps femoris* (FLAT: n=120) from cow carcasses. In the FLAT, tenderization method had no effect of MFI, TBA, or initial tenderness, sustained tenderness, or overall acceptability ($P > 0.05$). Enzymatic treatment affected all sensory attributes ($P < 0.05$); the control was the juiciest and the toughest with the least off flavor and the highest overall acceptability. Treatment affected TBA values; the bromelain and ficin treatment (BF) was most oxidized. Treatment by tenderization method interactions affected off flavor with BF having the most off flavor. In the PEC, treatment had affected MFI, cook loss, and all other sensorial evaluations ($P < 0.05$). However, TBA was not affected by treatment, tenderization method, or treatment by tenderization method. Moreover, MFI, juiciness, tenderness, and flavor intensity were all affected by tenderization method ($P < 0.05$).

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Product enhancement has long been a key area of concentration for beef research. Innovations in the beef industry have the potential to obtain greater consumer appeal, thus resulting in increased revenue for the packer, purveyor and ultimately the producer (Calkins and Sullivan, 2007). These improvements could vastly affect the overall acceptability of non-fed beef, as it is known for having lower palatability scores when compared to young, commodity beef (Purchas et al., 2002). The overall quality of meat is based on complex interactions that occur during a consumer's eating experience, also known as palatability (Huffman et al., 1996). In order to achieve optimum palatability, meat must be acceptable when considering tenderness, flavor, and juiciness, respectively in order of importance (Huffman et al., 1996). Non-fed beef tends to score lower in all 3 areas that contribute to palatability (Stelzleni et al., 2007; Moss and Calkins, 2007; and Faustman et al., 1996) thus resulting in lower consumer acceptability scores, which ultimately yield lower merchandizing value. Therefore, if any of these issues were combated with technological advances, it stands to reason that consumer acceptance would increase thus increasing merchandizing value.

The cause of such vast differences between commodity beef and non-fed beef can be explained by evaluating basic components of animal tissue. Components of concern include collagen, fat, and myoglobin as these elements become increasingly detrimental as animals mature. Collagen levels become an unfavorable issue in older cattle due to increased cross-

linking, which is directly related to decreased tenderness scores when evaluated by sensory panelists (Lawrie, 1998). In addition to inferior tenderness scores, non-fed beef scores lower when considering overall flavor acceptability as a result of a primarily forage diet leading to differing lipid composition (Stelzleni et al., 2007) as well as oxidation concerns from increased iron in myoglobin (Brewer, 2006). Furthermore, increased myoglobin results in darker, less desirable tissue color which leads to reduced merchandizing value due to lower consumer appeal (Faustman et al., 1996).

The oldest known technique utilized to improve tenderness and quality is a process known as ageing (Koohmarie, 1994). Ageing is a process in which a carcass or cut is held in a refrigerated environment for an extended period of time thus allowing ultrastructure degradation as result of endogenous enzymes (Aberle et al., 2001). However, aging alone cannot combat the tenderness issues found in non-fed beef as connective tissue within muscles from the round and chuck is far too abundant (Mandell et al., 2006 and Stelzleni et al., 2008). Nonetheless, current technology allows for improvement with interventions including mechanical tenderization (Maddock, 2008) and marinades (Calkins and Sullivan, 2007). Mechanical tenderization has proven to have a direct effect on tenderness scores, showing clear differences when utilized in commodity beef systems (Maddock, 2008). In addition to mechanical tenderization, chemical tenderization introduced through marinade systems can also affect tenderness scores. Not only can marinades grant opportunities for enzymatic tenderization but also allow for the addition of flavor components, increased water holding capacity, shelf-life, and microbial interventions (Calkins and Sullivan, 2007).

Enzymatic tenderization has proven to be successful when utilized on young, commodity beef in a study conducted by Calkins and Sullivan (2007). Calkins and Sullivan (2007) utilized enzymes from papaya, pineapple, and fig as well as enzymes sourced from bacteria and fungi.

In current research, enzyme treatments include combinations of bromelain, ficin, papain, and *bacillus* protease and were included at rates in compliance with the Code of Federal Regulations (part 21CFR184.1 – Direct Food Substances Affirmed as Generally Recognized as Safe by The United States Department of Agriculture's Food Safety Inspection Service) for use in food without limitation when good manufacturing practices are used. In combining methods of processing by utilizing both mechanical and chemical tenderization, possibilities of finding solutions to tenderness issues found in non-fed cattle can be improved. Therefore, non-fed beef has potential to gain increased consumer acceptance thus positively affecting the meat industry as a whole.

The major objective of this study was to evaluate combinations of exogenous enzymes introduced into a processing system in order to achieve optimum palatability of various cutlets from non-fed beef. Additionally, optimum combinations of enzymes paired with physical tenderization were evaluated using various analyses in order to measure tenderness and overall acceptability.

LITERATURE REVIEW

Non-fed Beef

According to the United State Department of Agriculture (2011), the contribution of cull cattle to the American beef supply is on the rise as evidenced by an increase of culled beef and dairy cattle, 4.86 million head in 2005 to 6.5 million head in 2010 or a four percent increase over a span of five years. While non-fed beef is predominantly a by-product of an industry focused on ultimately producing young, grain-fed cattle, there is monetary value to be gained from these animals (Stelzleni et al., 2007). Non-fed beef can be integrated into the food supply based on factors including poor performance regarding reproduction, insufficient milk production, or decreased growth efficiency due to age, genetic disposition, or medical issues including mastitis, lameness, Johne's disease, and bovine viral disease (BVD) (Wells et al., 1998). These culled animals contribute between 15-20 percent of a beef cattle producer's annual revenue (National Cattlemen's Beef Association, Beef Quality Audit GMPs, 1999). Older cattle are not as desirable as younger heifers and steers as they have proven to score lower when undergoing sensory and color evaluations. In total, there are few differences between old and young cattle, however, the enormity of these differences is prominent as the price of cow and bull beef is distinctly apparent when compared to young beef.

Sensory Attributes and Consumer Acceptance

Sensory panels are used to estimate consumer acceptability utilizing systems that reflect scores given to samples based on color and palatability (tenderness, juiciness, and

flavor). A study by Huffman et al. (1996) indicated that tenderness, flavor, and juiciness were rated respectively by consumers in order of importance when gauging overall palatability. When compared to young beef, sensory characteristics from non-fed cattle have scored markedly lower, reflecting lower consumer satisfaction, which in turn yields lower merchandizing value. Age is the primary explanation for these differences as non-fed beef consist of cattle that have been culled due to production in-efficiency. Therefore, non-fed beef poses issues of marketing concern since they are often older animals, which are known to have inferior tenderness, flavor, and color attributes (Lawrie, 1998). According to Lawrie (1998), as an animal gets older there is additional cross-linking of connective tissue within the muscles causing a greater amount of force to be used during consumption, thus resulting in a tougher, chewier piece of meat and consequently lower tenderness scores. Research done by Purchas et al. (2002) found that non-fed bull beef was significantly less tender than beef from a steer when considering all four aspects that comprise tenderness: hardness, cohesiveness, toughness, and chewiness. In agreement, Stelzleni et al. (2007) found non-fed beef and non-fed dairy beef to score inferior when assessing overall tenderness scores and flavor scores when compared to grain-fed cattle from both dairy and non-dairy circumstances. Stelzleni et al. (2007) also concluded the lack of tenderness was due to an increase of connective tissue that is not readily solubilized by heat, therefore not breaking down during most cooking techniques.

Second in priority for consumers is the flavor of the product. Meat flavor is a compilation of basic tastes and odors that are derived from a multitude of heat-activated chemical compounds found in all components of meat including lipids (Brewer, 2006).

Lipids are found in the different fats (subcutaneous fat, intermuscular fat, and intramuscular fat) throughout the carcass; since non-fed cattle are typically fed a forage diet as opposed to a high-energy grain diet, carcasses express less subcutaneous fat and less intramuscular fat (Stelzleni et al. 2007). Moreover, Brewer (2006) suggests that oxidation initiated by the iron found in myoglobin can affect off flavors in older cattle used for beef. Moss and Calkins (2007) found that a consumer panel used to detect off flavors in cow steaks characterized 30% of the samples (n=10) to have "bloody notes" and 10-20% of the samples found "livery and metallic flavor notes". As a result of these studies it can be concluded that maturity of a carcass can affect flavor through altered lipid profiles and increased iron intensity.

In addition to tenderness and flavor, color is an essential factor influencing consumer's willingness to purchase products and is also negatively affected due to enhanced age as animal maturity and lean color have a direct relationship. According to Faustman et al. (1996) animal maturity impacts lean color as concentrations of myoglobin are elevated in correspondence to age. The heme protein that is found in myoglobin is responsible for tissue colors, thus causing the muscle to appear darker as age increases (Faustman et al., 1996); this is significant as meat color is vitally important to consumer acceptance of a product. Appeal diminishes with color deterrence of the product from the bright-cherry red that is ideal in a retail setting (Cross et al., 1978).

Effects of Collagen on Tenderness

According to Weston et al. (2002) a direct link is found between collagen and tenderness variation in old beef. Collagen is the most abundant protein in the animal kingdom as its

purpose to assist animal tissues with elongation (Berk 2002). Being an integral addition to tendons and membranes, fibrils are a basic structure of collagen which consists of networking strands that are interweaved within one another and are responsible for the stability of the connective tissue (Neuman and Logan, 1950). Berk (2000) explains that all types of collagen found in the body eventually manifest a triple stranded structure; therefore interweaving, or cross-linking, plays a role in all types of collagen found within the body. In early life, these complex structures are reducible, but as maturing continues the cross-links are replaced by mature, thermally stable, less soluble collagen (Weston et al., 2002). It is important to note that these mature structures are the key factors in collagen-related tenderness and not the amount of collagen present (Weston et al., 2002). The primary problem concerning collagen is how it affects cooked meat. Overall, the ability of the triple helix structure to stabilize muscle fibers, as well as fibrils shrinking when heated result in water loss and ultimately decreased tenderness (Weston et al., 2002). However, research shows that the alteration of diet can increase the tenderness in older cattle. By feeding cattle a high grain/high energy diet collagen turnover rates increase resulting in a greater amount of unstable collagen cross-links are found within the muscle (Swatland, 1995). Collagen is in many ways responsible for the lack of tenderness in old beef; however, it is important to realize the role collagen plays in maintaining acceptable texture (Weston et al., 2002). Consequently, post-mortem collagen degradation must be managed carefully as mushy meat is also unacceptable to consumers (Huffman et al., 1996).

Processing Methods

The negative characteristics pertaining to non-fed beef as well as older cattle can be addressed with various interventions, including chemical and mechanical alterations. The abundance of technology that is applicable to underutilized cow and bull meat in order to achieve more desirable products has proven to be effective in young, commodity beef. Studies show that following mechanical and chemical tenderization methods, cuts from the round and chuck have potential to be used as foodservice steaks (Elam et al., 2002). These products are moderately priced as they fall between a high quality steak and product made from ground beef. Currently, enhanced goods derived mostly from underutilized cuts of the round and chuck of young commodity beef have proven successful in the foodservice sector as shown by an increasing numbers of providers, escalating from 1,000 retailers in 2001 to 9,000 retailers in 2006 (Beef Innovations Group, 2009). Innovations from the chuck include the Petite Tender (*Teres major*) and the extremely popular Flat Iron steak (*Infraspinatus*), which sold 92 million pounds in 12 months ending in August 2006 (Beef Innovations Group, 2009). The round provides 2 different Sirloin Tip cuts from the knuckle (*Quadriceps femoris,)* as well as the Western Griller and the Western Tip from the bottom round (*Biceps femoris)* (Beef Innovations Group, 2009). These value cuts are sometimes marinated or mechanically tenderized to achieve more desirable tenderness. The success of the mentioned value-added cuts found in young beef inspires potential for equivalent success of similar products from non-fed beef in an effort to endure economic urgency to increase revenue.

Tenderization Techniques

Mechanical Tenderization

In an effort to increase profit for the non-fed beef sector of the agriculture industry, the hindering aspects of culled cattle can be combated similarly to the underutilized cuts of young finished cattle. Tenderness being the principal limitation for older cattle has provided motivation for a multitude of research in anticipation for solutions to counteract the effects of a maturing animal. Interventions to improve tenderness include two primary means of mechanical tenderization; needle or blade tenderization and maceration or cubing. Needle or blade tenderization is accomplished by a set of needles or blades that penetrate the meat and cut muscle fibers and connective tissue, whereas maceration or cubing uses a series of small blades on rollers that macerate the surface of the meat that is passed through the rollers causing the texture and appearance of the product to be altered (Maddock, 2008). These tenderization methods can be and are used on portions of the carcass other than tender cuts such as the middle meats. In a study conducted by Smith et al. (1979), steaks ran twice through a blade tenderizer had significantly increased tenderness scores reported by sensory panel evaluations and Warner-Bratzler Shear Force analysis.

Enzymatic Tenderization

In addition to mechanical tenderization, marination or chemical tenderization can be used to enhance palatability of non-fed beef. Marination not only improves tenderness but can also be implemented to enhance juiciness and flavor via absorption/osmosis or by way of injection of a marinade into meat products thus affecting flavor, yields, water holding

capacity, shelf-life, and anti-microbial attributes (Brooks, 2007), providing a more profitable product. Marinades often include exogenous (introduced) enzymes that originate in fruits (papaya, pineapple, and fig), bacteria (*Bacillus subtilis)*, and fungi (*Aspergillus oryzae)* sources (Calkins and Sullivan, 2007). The papaya fruit produces the enzyme known as papain. Papain is aggressively destructive to both myofibrillar (attacks the z-line of the sarcomere) and collagen proteins and is much more effective when injected into a product (Calkins and Sullivan, 2007). Bromelain is derived from pineapples and is also damaging to both myofibrillar and collagen components of the muscle ultrastructure (Calkins and Sullivan, 2007). Ficin, an enzyme extracted from figs, is the least aggressive to all protein types when compared to plant derived enzymes (Calkins and Sullivan, 2007). In addition to exogenous enzymes, inherent (native) enzymes are used in the aging process. Aging is the oldest and simplest way to boost tenderness, mostly through sarcoplasmic protein degradation as the z-lines, costameric proteins, and titan are attacked by the calpain system (Calkins and Sullivan, 2007).

Solutions such as mechanical, chemical and enzymatic tenderization allow for a variety of procedures that can be effective in resolutions of tenderness, flavor, and shelf-life issues; this in turn yields a higher profit for the entire non-fed beef sector. The majority of the monetary turnover is from the "middle meats" as they are sold as wholesale ribs, loins, and sirloins and cut into steaks (NCBA Beef Quality Audit GMPs 1999). However, cuts that could be processed and sold at a higher price than ground product would easily result in a higher profit ensuring more money for all portions of the market cow and bull system.

Future of Value-Added Products

Considering the economic pressure that continues to affect all sectors of the beef industry, there is more of a demand for value-added products and opportunities especially in the processed meats industry. With the addition of additives and innovative processing, off-flavors and tenderness found in older cattle can be remedied. Consumers that purchase these products are patrons who are loyal to beef and appreciate the nutritional value but cannot always afford high quality and high priced steaks. However with these enhanced products the consumer could feel as if they consumed a quality meal for a reasonable price. With a processed product such as the intermediate cuts, the capability to reproduce the products is much easier and is much more reliable. Repeatability and consistency keep the foodservice sector at ease and the customer fulfilled, as they both can rely on a familiar product. Therefore, much can be gained from enhancing cow and bull beef as tenderness, flavor, and juiciness play an imperative role in consumer acceptance. Product consistency and consumer satisfaction will potentially result in a constant demand for enhanced products. Furthermore, improved product yields in conjunction with the improved consumer appeal and ultimately consumer satisfaction could result in higher merchandizing value for older culled non-fed cow and bull beef.

MATERIALS AND METHODS

Treatments

Cow carcasses (n=120) from a commercial non-fed abattoir within 16.09 km (10 miles) of the Angelo State University Food Safety and Product Development Laboratory (FSPD) in San Angelo, TX were evaluated according to the predetermined selection criteria, having a skeletal maturity of C^{50} – D^{50}. Carcasses were tagged for identification and the pectoral (NAMP# 115D – Deep Pectoral) and the bottom (gooseneck) round (NAMP# 171BFL06) were collected 24 hours postmortem (PM), denuded and transported to the FSPD. Samples were placed in a 4°C cooler under vacuum package storage for approximately 4 days. The bottom round was fabricated on day 6 PM (6d-PM) into the outside round/flat (NAMP# 171BL06 or *biceps femoris* and may contain *gluteus medius*, *gluteus profundus*, and *gluteus accessories*). Muscles (n=120) were randomly assigned to 1 of 6 enzyme treatments (n=20/enzyme treatment; Table 1). Enzymatic treatments were as follows: BBA = Bromelain and *Bacillus*, BF = Bromelain and Ficin, BP = Bromelain and Papain, C = Control, PBA = Papain and *Bacillus*, and FP = Ficin and Papain.

Muscles were then injected using a multi-needle injector (KOCH günter pökelinjektor – Kansas City, MO) and vacuum tumbled (KOCH LT-15 – Kansas City, MO) for 20 minutes. Base brine was formulated consisting of water, sodium phosphate, sodium chloride, and calcium chloride and muscles were pumped to 7.5% of green weight (Table 1). Ingredient concentration in the brine was such that muscles contained 0.25% sodium phosphate, 0.5% sodium chloride, and 0.25% of 250 mM calcium chloride after marination.

Following marination, samples within an enzyme treatment were then assigned to tenderization treatments consisting of mechanical tenderization or non-mechanically tenderized control (n=10/enzyme, marination and tenderization combination: Figure 1). Samples designated as mechanically tenderized were run twice at a 90° angle thru a mechanical tenderizer (BIRO Pro-9). After muscle treatment assignment, muscles were then portioned by the muscle being cut beginning at the most anterior point and serially cut into 2.54cm (1 in.) slices (approximately 6 slices per muscle portion). Slices were then serially assigned to myofibril fragmentation index (MFI; 1 slice), thiobarbituric acid assay (TBA; 1 slice), trained sensory panels (2 slices), and other analysis (2 slices). Samples were frozen at -10°C before being analyzed for MFI, TBA, and sensory.

Myofibrillar and Connective Tissue Tenderization Evaluation

Myofibril Fragmentation Index

Cutlets were removed from frozen storage and thawed at 4°C 12 – 24 hours prior to MFI analysis. Cutlet samples were analyzed for myofibril fragmentation according to procedures as outlined by Culler et al. (1978). During the extraction phase of MFI analyses, 4 grams (g) of muscle were blended with 40 milliliters (mL) of cold (2°C) MFI buffer solution in duplicate for 30 seconds and then centrifuged at 1,000 X G for 15 minutes. After discarding the supernatant, the pellet was suspended for a second time in MFI buffer and centrifuged again at 1,000 X G for 15 minutes. Next, the supernatant and the fat cap were discarded and the pellet was suspended once more in MFI buffer. Following the extraction phase, the protein assay phase was conducted using the final suspensions. The protein assay

phase began by combining 0.25 mL of each suspension with 0.75 mL MFI buffer and 4 mL biuret reagent. After vortexing, the mixture was placed in a dark at room temperature (20-25°C) for 30 minutes. Protein concentrations were determined using absorbance values of 540 nm using a spectrophotometer (Thermo Spectronic: Genesys 20). MFI measurements were determined by combining the suspensions and MFI buffer to make 8 mL of a 0.5 mg protein/mL solution, and after homogenizing, the solution was read at 540 nm. MFI values were taken after multiplying the spectrophotometer reading by 200 (Culler et al., 1978).

Sensorial Evaluation

Prior to beginning of evaluations, panelist were trained in accordance to procedures of Cross et al. (1978) in order for trainees to comprehend testing procedures as well as evaluation of product. Cutlets were removed from frozen storage and thawed under refrigeration at 4°C for 24 hr prior to sensory evaluation. Cutlets were cooked on an electric clam-shell-style grill to an internal temperature of approximately 71°C to achieve a medium degree of doneness according to procedures outlined by Kerth et al. (2003). Cutlets were then cut into $1cm^3$ pieces and stored in warming pans until all samples for that panel were prepared. All samples were served warm to panelist within 15 minutes of cook time. A minimum of six trained panelist were used for sensory evaluation for each panel. Each panelist was provided apple juice and water to cleanse the pallet before each sample was evaluated. Cutlets were analyzed for initial and sustained juiciness, initial and sustained tenderness, flavor intensity, off flavor, and overall acceptability according to procedures of Cross et al. (1978; Table 2).

Oxidative Stability Assessment

Lipid oxidative stability was determined utilizing a thiobarbituric acid (TBA) reactive substance assay as detailed by Buege and Aust (1978). Lipid analysis assisted in determining any deleterious effects on product quality and shelf-life potentially caused by the respective treatments. TBA procedure was completed in triplicate and the average of three values was used for analysis. Samples were removed from frozen storage and placed under refrigeration of 4°C, 12 to 24 hours prior to oxidative stability assessment. Next, 10 g of sample was combined with 30 mL of distilled water and then blended to achieve the homogenate. Next, approximately 4 mL of homogenate was combined with 8 mL of trichloracetic/thiobarbituric acid reagent and 100µL of 10% butylatedhydroxyanisole. Samples were then incubated in a 99°C water bath for 15 minutes, allowed to cool in a cold-water bath for 10 minutes and spun at 2000 X G for 10 minutes. The absorbance was read against a blank containing like reagents at 531 nm on a spectrophotometer. Malonaldehyde standard, utilizing 1,1,3,3-tetraethoxypropane and thiobarbituric acid was used, and thiobarbituric acid substances were reported as mg/10g of meat.

Statistical Analysis

Data was analyzed as a 6 X 2 factorial arrangement (6 marination treatments X 2 mechanically tenderization treatments) of a completely randomized design using the general linear models procedures of SAS (SAS Inst. Inc., Cary, NC). All TBA, MFI, and sensory data were included in the model with treatment, tenderization, and treatment x tenderization

as fixed effects. Animal served as experimental unit and significant ($P \leq 0.05$) treatment effect means were separated using Fisher's protected LSD.

RESULTS AND DISCUSSION

Myofibril Fragmentation

Myofibrillar fragmentation (MFI) *Biceps* femoris (FLAT) values were not dependent on tenderization method ($P = 0.39$; Table 3). However, MFI values tended to be reliant upon enzymatic treatment ($P = 0.08$) and enzymatic treatment x tenderization method ($P = 0.09$). Enzymatic treatment of papain and *Bacillus* (PBA) MFI value tended to be greater than all remaining enzymatic treatments. When evaluating MFI values of deep pectoral (PEC), values were dependent on enzymatic treatment ($P < 0.001$) and tenderization method ($P = 0.02$) but were not reliant on treatment x tenderization method ($P = 0.63$; Table 3). Enzymatic treatment containing bromelain and papain (BP) had the highest MFI value with PBA having the second highest MFI value. Mechanical tenderization had the higher MFI value of the two tenderization methods ($P = 0.02$). Therefore, samples treated with BP, BPA, or a mechanical tenderization method indicated increased myofibrillar fragmentation in pectoral muscle samples.

Meat tenderness is an intricate attribute when considering beef palatability as meat tenderness is in direct correlation with sex, age, and diet of the animal as these factors affect complex biochemical processes. Fibrils, fibers, and filaments found within the muscle provide an integral role in maintaining structure within the muscle. Thus, evaluations of these components are utilized in determining effects of protein degradation as a gauge of tenderness. These processes vary in specific procedure as evidenced by different methods evaluating myofibril fragmentation via protein concentrations (Culler et al., 1978), weight of filtered sample (Purchas et al., 1997), and measurement of fragment length (Fernandez and

Tornberg, 1994). MFI measures in this study showed treatments utilizing papain tended to be successful in the FLAT and were more affective in the PEC. These findings could be explained by a study by Strandine et al., (1949) that found a greater amount of collagen in the PEC. In addition, these results are consistent with a study done by Calkins and Sullivan (2007) which concluded that papain was the most aggressive enzyme when compared to bromelain and ficin when integrated into a meat system utilizing the *supraspinatus* (high levels of connective tissue) and the *triceps brachii* (low levels of connective tiusse) . As expected, the control enzyme treatment had the least myofibrillar fragmentation as there were no exogenous enzymes in these samples. BBA in the PEC and BF in the FLAT had some of the lowest MFI values of the enzyme combination treatments (118.13 ± 7.49 and 121.31 ± 6.22; respectively). These results are consistent with information provided by Tarté (2009) that explains the similarity of activity and behavior of ficin and *Bacillus* as BBA treatments contain *Bacillus* and BF treatments contain ficin. Bromelain has proven to be active on both intracellular (myofibrillar) and extracellular (collagen) protein, thus suggesting that the bromelain in BBA and BF treatments is active in these systems (Calkins and Sullivan, 2007). Furthermore, as expected, the PEC samples that were mechanically tenderized had more myofibrillar fragmentation, as expected.

Sensory

Biceps femoris (FLAT)

Sensory attribute scoring based on enzymatic treatment of FLAT is denoted in Table 4 and sensory attributes based on mechanical tenderization method of FLAT are reported in Table 5. Cook loss values were only dependent on mechanical tenderization method ($P < 0.001$) with non-mechanically tenderized samples having the least amount of cook loss. No differences were found due to enzymatic treatments ($P = 0.14$) or treatment x tenderization method ($P = 0.22$). Initial juiciness values were affected by enzymatic treatment ($P < 0.001$) as well as tenderization method ($P < 0.001$) with samples in the control enzymatic treatment having the highest initial juiciness and the ficin and papain (FP) treatment having the lowest initial juiciness among enzymatic treatments. Also, samples that were not mechanically tenderized had the highest initial juiciness between the two mechanical tenderization methods ($P < 0.001$). Moreover, initial juiciness values tended to be subject to treatment x tenderization ($P = 0.069$) interaction as control x no tenderization (C x NO) tended to be the juiciest and FP x mechanical tenderization (FP x MT) tended to be the least juicy when evaluated by panelist. Sustained juiciness values were affected by enzymatic treatments ($P < 0.001$), and tenderization method ($P < 0.001$), but not by enzymatic treatment x tenderization method ($P = 0.23$). Control samples had the highest sustained juiciness within enzymatic treatment and samples that were not mechanically tenderized had higher sustained juiciness between the two tenderization methods. Initial tenderness values were reliant on enzymatic treatment ($P < 0.001$) as control samples were the least tender. Conversely, tenderization method ($P = 0.13$) or enzymatic treatment x

tenderization ($P = 0.15$) had no affect on initial tenderness ratings. Sustained tenderness values were dependent on enzymatic treatments ($P < 0.001$), as control samples had the lowest tenderness rating, but values were not reliant on tenderness method ($P = 0.12$) or enzymatic treatment x tenderness method ($P = 0.25$) interaction. Beef flavor intensity ratings were subject to enzymatic treatment ($P < 0.001$) and tenderness method ($P = 0.003$) with control samples (6.40 ± 0.10) having the most intense beef flavor across enzymatic treatments ranking half a point higher than all remaining enzymatic treatments with BBA (5.90 ± 0.10) being at the top of the remaining enzyme treatments. Samples with no mechanical tenderization ranked as the most intensely flavored between the two tenderization methods ($P = 0.003$). However, no dependence was evident on enzymatic treatment x tenderization method ($P = 0.44$). Off flavor ratings values were dependent on enzymatic treatment x tenderness method ($P = 0.02$; Table 7) with the control treatment with mechanical tenderization (C x MT) having samples with the least off flavor (4.00 ± 0.03) and the bromelain and ficin (BF) treatment with mechanical tenderization (BF x MT) having samples with the most off flavor (3.82 ± 0.03). Overall acceptability values showed dependence on enzymatic treatment ($P < 0.001$), as sample from the control enzyme treatment had the most acceptable (5.98 ± 0.21) ranking over a full point higher than the bromelain and *Bacillus* (BBA) enzyme treatment (4.90 ± 0.21); samples from all remaining enzyme treatments were less acceptable. Furthermore, overall acceptability values tended to be subject to tenderization method ($P = 0.08$) as no tenderization was the most acceptable when evaluated by panelist. Overall acceptability ratings were not dependent on enzymatic treatment x tenderization method ($P = 0.22$).

Deep Pectoral (PEC)

Sensory attributes based on enzymatic treatment of PEC are denoted in Table 7 and sensory attributes based on tenderization method of PEC are in Table 8. Cook loss values were dependent on enzymatic treatments ($P < 0.001$) as samples from the control enzyme treatment had the least amount of cook loss. However, values showed no reliance on tenderization method ($P = 0.24$) or enzymatic treatment x tenderization method ($P = 0.66$). Initial juiciness values were dependent on enzymatic treatments ($P = 0.004$) as well as tenderization method ($P < 0.001$), as control samples were the juiciest across enzymatic treatments and samples that were not tenderized were juicier. However, there was no reliance on interaction among enzymatic treatment x tenderization method ($P = 0.91$) when evaluating initial juiciness ratings. Sustained juiciness values showed dependence on enzymatic treatment ($P < 0.001$) and tenderization method ($P = 0.002$), as control samples were the juiciest and samples in the bromelain and ficin (BF) treatment were the least juicy of the enzymatic treatments. Also, samples with no tenderization were the juiciest when comparing the two tenderization methods. Sustained juiciness values showed no reliance due to enzymatic treatment x tenderization method ($P = 0.96$). Tenderness affects were dependent on enzymatic treatment ($P < 0.001$) as well as tenderization method ($P < 0.001$) and tended to be subject to enzymatic treatment x tenderization method ($P = 0.07$) when measuring initial tenderness values. Control samples from the enzymatic treatment were the least tender with all remaining enzymatic treatment scoring within one point of each other ranging from 4.97 to 5.90 ± 0.19. Additionally, mechanically tenderized samples were more tender than samples that were not tenderized ($P < 0.001$). Furthermore,

samples that were in the treatment containing ficin and papain (FP) by mechanically tenderized (FP x MT) as well as samples in treatment PBA by mechanically tenderized (PBA x MT) tended to be the most tender when comparing interactions of enzymatic treatment x tenderization method as the both scored 6.30 ± 0.27. Sustained tenderness values were dependent on enzymatic treatments ($P < 0.001$) as well as tenderization method ($P < 0.001$) and tended to be subject to enzymatic treatment x tenderization method ($P = 0.08$); the control was again the least tender, ranking more than half a point behind all remaining enzymatic treatments, and mechanical tenderization was once more the more tender of the two tenderization methods. However, samples in the treatment BP that were mechanically tenderized (BP x MT) tended to be more tender than samples in all other enzymatic treatment and tenderization method combinations. Beef flavor intensity values were affected by enzymatic treatment ($P < 0.001$) as well as tenderization method ($P < 0.001$); control samples had the most intense flavor, and samples in the treatment containing bromelain and *Bacillus* (BBA) as well as (BP) samples both had the least intense beef flavor. Also, samples that were not mechanically tenderized had a more intense beef flavor than samples that were mechanically tenderized. Flavor intensity values were not subject to enzymatic treatment x tenderization method ($P = 0.55$). Off flavor values were not reliant on enzymatic treatment ($P = 0.40$), tenderization method ($P = 0.95$), or treatment x tenderization method ($P = 0.28$). Overall acceptability values in the pectoral muscle were subject to enzymatic treatment effect ($P < 0.001$), with the control sample being markedly more acceptable over all enzymatic treatments, with BBA being the second most acceptable of the remaining enzymatic treatments and FP being the least acceptable. Overall

acceptability ratings were not affected by tenderization method ($P = 0.29$) or enzyme treatment x tenderization method ($P = 0.73$).

Palatability of beef products is the most important characteristic to the majority of consumers as tenderness, juiciness, and flavor all provide an integral role in the acceptance of beef products. Unfortunately, beef from cow and bull carcasses has always deterred consumer's palates (Woerner, 2010) as there are notable differences in tenderness, juiciness, and flavor. However, as technology improves the beef industry's ability to combat these hurdles improves as well. The results of this study show the capability of endogenous enzymes, as well as mechanical tenderization, to modify sensorial attributes of cow beef. In the FLAT and the PEC, the lack of mechanical tenderization explains why the control treatment was the juiciest as there were no physical scores on the surface of the meat thus containing moisture within the sample. Also, the lack of mechanical tenderization coupled with the absence of exogenous enzymes could explain why the control treatment was determined to be the least tender in the FLAT as well as the PEC. It is important to note that FP, PBA, and BP all averaged higher than a 6 (extremely tender), approaching a 7 (moderately over tender) thus raising a concern for a potentially over tender product. Also, these three treatments all contain papain, again showing consistency with its aggressive tendencies that have been documented by Tarté (2009) and Calkins and Sullivan (2007). In addition, the toughest samples outside of the control treatment for each muscle contained bromelain and *Bacillus* which is consistent with findings reported by Calkins and Sullivan (2009) that found these two enzyme to be the least aggressive of the enzymes used in this study. Flavor intensity in both the FLAT and PEC were affected by enzyme treatment as

well as tenderization method as control treatments scored the highest in both muscles and the three treatments that had the least flavor intensity each contained papain which is sometimes responsible for a bitter aftertaste (Tarté, 2009). In the FLAT, off flavor was highest in BF x MT, which is inconsistent with other studies that found ficin to have little bitter flavor and bromelain to have little or no off flavor (Tarté, 2009). The affect of liplolytic and/or proteolytic enzymes on lipolysis and proteolysis and the interaction with flavor components in meat still needs to be studied further (Toldrá et al., 2005). The control treatment in the FLAT was the most acceptable but scoring only a 6 (Like Moderately) with four of the five remaining treatments averaging a 3 (Dislike Moderately) when evaluated for overall acceptability. In the PEC, the control treatment was the most acceptable averaging a 5 (Like Slightly) with four of the five remaining treatments averaging a 4 (Dislike Slightly) when evaluated for overall acceptability.

Oxidative Stability

Thiobarbituric reactive substances (TBA) values for the FLAT muscle were dependent on enzymatic treatments ($P = 0.04$; Table 9), as BF contained the most oxidized substances. However, TBA values were not subject to tenderization method ($P = 0.13$) or treatment x tenderization method ($P = 0.35$). In addition, TBA values for the PEC were not dependent on enzymatic treatments ($P = 0.12$; Table 9), tenderization method ($P = 0.99$), or enzymatic treatment x tenderization method ($P = 0.89$).

Oxidation is utilized to estimate the shelf-life as lipid oxidation contributes to off flavor and product deterioration of meat products. Shelf-life is the amount of time a

product can be stored before quality and safety of the product begin to diminish. In this study it was found that oxidation was dependent on enzyme treatment. The high oxidation values of the treatment containing bromelain and ficin may be due to the low inactivation temperature of ficin potentially active at refrigeration temperatures.. With a low activation rate, it is possible that the enzymes were not kept cold enough during sample preparation for TBA analysis and ficin became active during the hold time (Tarté, 2009). However, the lack of oxidation in the other treatments in both the PEC and the FLAT could suggest that enzyme inclusion does not reduce shelf-life. This is important to consider as this product is aimed at the food service industry, thus allowing for a longer storage period than fresh cuts sold in the grocery store. However, it is important to note that bromelain has been noted by Calkins and Sullivan (2007) to be minimally active at temperatures as low as 0°C.

IMPLICATIONS

The overall palatability of a meat product must be acceptable in order for the product to be viable in the market place. Exogenous enzymes are currently utilized in commodity (young) beef system in order to improve tenderness. Achieving the same success in non-fed (older) beef systems has the potential to positively affect all segments of the cattle industry. All cutlets that were applied with combinations of exogenous enzymes showed increased tenderness. Moreover, the results of this study do not show that overall acceptability of non-fed beef cutlets to have improved overall acceptability. With the adjustment of enzyme inclusion rates, producers could include enzymes in non-fed beef product systems and affect not only the FLAT and PEC, and potentially other muscles that are known for being tough. Further research is necessary in order to determine specific affects of papain, bromelain, ficin and *Bacillus* protease on non-fed beef. As there are a variety of exogenous enzymes, combinations of these enzymes as well as individual enzyme interaction should be further evaluated. Trials focused on non-fed beef interaction with specific exogenous enzymes would give the beef industry a better understanding of how these enzymes interact with high levels of collagen. Furthermore, consumer acceptability has not yet been evaluated and needs to be addressed before alterations or implementation occurs.

Table 1. Brine Formulation

Base Brine[a]		
Ingredients[b]	**Amount in Brine (kg)**	**% in Product**[c]
Sodium Chloride	1.20	0.50
Sodium Phosphate	0.60	0.25
Calcium Chloride	0.60	0.25

Treatment[d]	**Enzyme Amounts in Brine**[a] **(g)**	**% in Product** [b]
1	Bromelain-154.00 + Ficin-122.00	Bromelain-0.000632/Ficin-0.000496
2	Papain-123.00 + *Bacillus*-107.00	Papain-0.000551/*Bacillus*-0.000496
3	Bromelain-154.00 + Papain-123.00	Bromelain-0.000632/Papain-.0000551
4	Ficin-122.00 + Papain-123.00	Ficin-0.000496/Papain-0.000551
5	Bromelain-154.00 + *Bacillus*-107.00	Bromelain-0.000632/*Bacillus*-0.000496
6	Control	Control

[a] Base brine was included in all treatments along with respective enzyme combinations
[b] All formulations are based on a 18.14 kg brine solution
[c] All product were pumped to 7.5% of green weight
[d] Treatments 7-12 are duplicates of treatments 1-6 with the addition of mechanical tenderization

Table 2. Sensory Panel Scoring for Non-fed Beef Cuts

Juiciness	Tenderness	Flavor Intensity
8 Extremely Juicy	8 Extremely Over Tender	8 Extremely Intense
7 Very Juicy	7 Moderately Over Tender	7 Very Intense
6 Moderately Juicy	6 Extremely Tender	6 Moderately Intense
5 Slightly Juicy	5 Moderately Tender	5 Slightly Intense
4 Slightly Dry	4 Slightly Tender	4 Slightly Bland
3 Moderately Dry	3 Slightly Tough	3 Moderately Bland
2 Very Dry	2 Moderately Tough	2 Very Bland
1 Extremely Dry	1 Extremely Tough	1 Extremely Bland

Off Flavor	Overall Acceptability
4 None	8 Like Extremely
3 Slight Off Flavor	7 Like Very Much
2 Moderate Off Flavor	6 Like Moderately
1 Extreme Off Flavor	5 Like Slightly
	4 Dislike Slightly
	3 Dislike Moderately
	2 Dislike Very Much
	1 Dislike Extremely

Table 3. LS Means ± SE of Myofibril Fragmentation Index (MFI) for Enzymatic Treatment and Tenderization Method on Pectoral (n=119) and FLAT Muscles (n=120)

Enzymatic Treatment[a]	**MFI FLAT**[b]	**MFI PEC**[c]
BBA	128.65 ± 6.26	118.13 ± 7.49^{vw}
BF	121.31 ± 6.22	145.57± 7.49^{yz}
BP	123.43 ± 6.22	164.81± 7.45^{z}
C	139.18 ± 6.22	107.71± 7.65^{v}
FP	136.94 ± 6.22	136.89± 7.45^{wx}
PBA	143.15 ± 6.22	161.73± 7.22^{yz}
Tenderization Method[d]		
NO	129.92 ± 3.57	131.46 ± 4.35^{y}
MT	134.29 ± 3.63	145.83 ± 4.27^{z}

[a] Enzymatic Treatments: BBA = Bromelain and *Bacillus*, BF = Bromelain and Ficin, BP = Bromelain and Papain, C = Control, FP = Ficin and Papain and PBA = Papain and *Bacillus*

[b] NAMP# 171BL06 or *biceps femoris* (Enzymatic Treatment: $P = 0.08$; Tenderization: $P = 0.39$; and Treatment x Tenderization: $P = 0.09$)

[c] NAMP# 115D or Deep Pectoral (Enzymatic Treatment: $P < 0.001$; Tenderization: $P = 0.02$; and Treatment x Tenderization: $P = 0.63$)

[d] NO = No Mechanical Tenderization or MT = Mechanical Tenderization

[zyxwv] Means within a muscle attribute combination that lack common superscripts differ ($P \leq 0.05$)

Table 4. LS Means ± SE of Sensory Attributes of Enzymatic Treatments for FLAT Muscle[a] (n=120)

Attribute	Enzymatic Treatments[b]						
	BBA	**BF**	**BP**	**Control**	**FP**	**PBA**	***P* > F**
CL[c]	129.72 ± 9.73	142.38 ± 10.15	114.50 ± 9.88	134.52 ± 9.65	142.05 ± 9.88	112.56 ± 10.26	0.14
IJ[d]	4.84 ± 0.14^{y}	5.15 ± 0.15^{y}	4.92 ± 0.14^{y}	5.86 ± 0.14^{z}	4.83 ± 0.14^{y}	5.15 ± 0.15^{y}	<0.001
SJ[e]	4.91 ± 0.15^{y}	5.17 ± 0.15^{y}	5.06 ± 0.15^{y}	5.86 ± 0.15^{z}	4.93 ± 0.15^{y}	5.12 ± 0.15^{y}	<0.001
IT[f]	5.55 ± 0.20^{xw}	6.20 ± 0.21^{zyx}	6.44 ± 0.21^{zy}	4.77 ± 0.20^{wv}	6.51 ± 0.21^{z}	6.06 ± 0.22^{zyxw}	<0.001
ST[g]	5.41 ± 0.22^{wv}	6.00 ± 0.23^{zyxw}	6.32 ± 0.22^{z}	4.96 ± 0.21^{v}	6.22 ± 0.22^{zy}	6.06 ± 0.23^{zyx}	<0.001
FI[h]	5.90 ± 0.10^{y}	5.60 ± 0.10^{x}	5.61 ± 0.10^{x}	6.40 ± 0.10^{z}	5.58 ± 0.10^{x}	5.44 ± 0.10^{x}	<0.001
OF[i]	3.96 ± 0.02	3.90 ± 0.02	3.95 ± 0.02	3.97 ± 0.02	3.91 ± 0.02	3.91 ± 0.02	0.16
OA[j]	4.90 ± 0.21^{y}	3.70 ± 0.22^{x}	3.77 ± 0.21^{x}	5.98 ± 0.21^{z}	3.55 ± 0.21^{x}	3.80 ± 0.22^{x}	<0.001

[a] NAMP# 171BL06 or *biceps femoris*

[b] Enzymatic Treatments: BBA = Bromelain and *Bacillus*, BF = Bromelain and Ficin, BP = Bromelain and Papain, C = Control, FP = Ficin and Papain and PBA = Papain and *Bacillus*

[c] Cook Loss (g)

[d] Initial Juiciness:1=Extremely Dry, 8=Extremely Juicy

[e] Sustained Juiciness:1=Extremely Dry, 8=Extremely Juicy

[f] Initial Tenderness:1=Extremely Tough, 8=Extremely Over Tender

[g] Sustained Tenderness:1=Extremely Tough, 8=Extremely Over Tender

[h] Flavor Intensity:1=Extremely Bland, 8=Extremely Intense

[i] Off Flavor:1=Extreme Off Flavor, 4 = None

[j] Overall Acceptability:1=Dislike Extremely, 8=Like Extremely

[zyxwv] Means within a attribute that lack common superscripts differ ($P \leq 0.05$)

Table 5. LS Means ± SE of Sensory Attributes of Tenderization Method for Round - FLAT Muscle[a] (n=120)

Attribute	NO[b]	MT[c]	*P* > F
Cook Loss (g)	113.88 ± 5.59	144.70 ± 5.87	0.002
Initial Juciness[d]	5.52 ± 0.08	4.73 ± 0.09	<0.001
Sustained Juiciness[e]	5.58 ± 0.08	4.77 ± 0.09	<0.001
Initial Tenderness[f]	6.05 ± 0.12	5.79 ± 0.12	0.13
Sustained Tenderness[g]	5.68 ± 0.13	5.96 ± 0.13	0.12
Flavor Intensity[h]	5.88 ± 0.06	5.63 ± 0.06	0.003
Off Flavor[i]	3.94 ± 0.01	3.92 ± 0.01	0.31
Overall Acceptability[j]	4.49 ± 0.12	4.67 ± 0.13	0.08

[a] NAMP# 171BL06 or *biceps femoris*
[b] NO = No Mechanical Tenderization
[c] MT = Mechanical Tenderization
[d] Initial Juiciness:1=Extremely Dry, 8=Extremely Juicy
[e] Sustained Juiciness:1=Extremely Dry, 8=Extremely Juicy
[f] Initial Tenderness:1=Extremely Tough, 8=Extremely Over Tender
[g] Sustained Tenderness:1=Extremely Tough, 8=Extremely Over Tender
[h] Flavor Intensity:1=Extremely Bland, 8=Extremely Intense
[i] Off Flavor:1=Extreme Off Flavor, 4 = None
[j] Overall Acceptability:1=Dislike Extremely, 8=Like Extremely
Means with corresponding values ($P < 0.05$) differ.

Table 6. LS Means ± SE of Off Flavor Scores for Enzymatic Treatment x Tenderization Method for FLAT Muscle[a] (n=120)

Enzyme Treatment	Mechanical Tenderization	No Tenderization
BBA	3.93 ± 0.03^{zyxwvut}	3.98 ± 0.03^{zy}
BF	3.82 ± 0.03^{t}	3.98 ± 0.03^{zxy}
BP	3.95 ± 0.03^{zyxw}	3.94 ± 0.03^{zyxwvu}
C	4.00 ± 0.03^{z}	3.95 ± 0.03^{zyxwv}
FP	3.90 ± 0.03^{xwvut}	3.92 ± 0.03^{zyxwvut}
PBA	3.94 ± 0.04^{zyxwvut}	3.89 ± 0.03^{wvut}

[a] NAMP# 171BL06 or *biceps femoris* ($P < 0.001$)

[b] Enzymatic Treatments: BBA = Bromelain and *Bacillus*, BF = Bromelain and Ficin, BP = Bromelain and Papain, C = Control, FP = Ficin and Papain and PBA = Papain and *Bacillus*

[c] NO = No Mechanical Tenderization or MT = Mechanical Tenderization

^{zyxwvut} Means that lack common superscripts differ ($P \leq 0.05$)

Table 7. LS Means ± SE of Sensory Attributes of Enzymatic Treatments for Pectoral Muscle[a] (n=119)

Attribute	Enzymatic Treatments[b]						
	BBA	**BF**	**BP**	**Control**	**FP**	**PBA**	***P* > F**
CL[c]	102.39 ± 8.51^{w}	135.67 ± 8.70^{zy}	140.30 ± 8.47^{z}	95.69 ± 8.51^{w}	134.95 ± 8.47^{zyx}	103.40 ± 8.47^{w}	<0.001
IJ[d]	5.33 ± 0.13^{zy}	5.18 ± 0.14^{yxw}	4.91 ± 0.13^{w}	5.57 ± 0.13^{z}	4.94 ± 0.13^{w}	5.33 ± 0.13^{zyx}	0.004
SJ[e]	5.46 ± 0.14^{yx}	5.33 ± 0.15^{yxw}	4.99 ± 0.14^{w}	5.88 ± 0.14^{z}	5.08 ± 0.14^{yxw}	5.51 ± 0.14^{zy}	<0.001
IT[f]	4.97 ± 0.19^{v}	5.76 ± 0.20^{zyx}	5.51 ± 0.19^{zyxw}	4.24 ± 0.19	5.89 ± 0.19^{zy}	5.90 ± 0.19^{z}	<0.001
ST[g]	5.31 ± 0.19^{v}	6.05 ± 0.19^{zyx}	5.96 ± 0.19^{zyxw}	4.48 ± 0.19	6.19 ± 0.19^{zy}	6.21 ± 0.19^{z}	<0.001
FI[h]	5.57 ± 0.09^{y}	5.35 ± 0.09^{yx}	5.31 ± 0.09^{x}	5.97 ± 0.09^{z}	5.36 ± 0.09^{yx}	5.31 ± 0.09^{x}	<0.001
OF[i]	3.90 ± 0.04	3.85 ± 0.04	3.94 ± 0.04	3.97 ± 0.04	3.90 ± 0.04	3.94 ± 0.04	0.40
OA[j]	4.84 ± 0.20^{y}	4.03 ± 0.21^{w}	4.15 ± 0.20^{xw}	5.77 ± 0.20^{z}	3.98 ± 0.20^{w}	4.70 ± 0.20^{yx}	<0.001

[a] NAMP# 115D or Deep Pectoral

[b] Enzymatic Treatments: BBA = Bromelain and *Bacillus*, BF = Bromelain and Ficin, BP = Bromelain and Papain, C = Control, FP = Ficin and Papain and PBA = Papain and *Bacillus*

[c] Cook Loss (g)

[d] Initial Juiciness:1=Extremely Dry, 8=Extremely Juicy

[e] Sustained Juiciness:1=Extremely Dry, 8=Extremely Juicy

[f] Initial Tenderness:1=Extremely Tough, 8=Extremely Over Tender

[g] Sustained Tenderness:1=Extremely Tough, 8=Extremely Over Tender

[h] Flavor Intensity:1=Extremely Bland, 8=Extremely Intense

[i] Off Flavor:1=Extreme Off Flavor, 4 = None

[j] Overall Acceptability:1=Dislike Extremely, 8=Like Extremely

[zyxwv] Means within a attribute that lack common superscripts differ ($P \leq 0.05$)

Table 8. LS Means ± SE of Sensory Attributes of Tenderization Method for Pectoral Muscle[a] (n=119)

Attribute	NO[b]	MT[c]	*P* > F
Cook Loss (g)	114.65 ± 4.86	122.82 ± 4.98	0.24
Initial Juciness[d]	5.40 ± 0.08	5.01 ± 0.08	0.001
Sustained Juiciness[e]	5.57 ± 0.08	5.18 ± 0.08	0.002
Initial Tenderness[f]	4.84 ± 0.11	5.91 ± 0.11	<0.001
Sustained Tenderness[g]	5.16 ± 0.11	6.24 ± 0.11	<0.001
Flavor Intensity[h]	5.63 ± 0.05	5.33 ± 0.05	<0.001
Off Flavor[i]	3.92 ± 0.02	3.92 ± 0.02	0.95
Overall Acceptability[j]	4.49 ± 0.12	4.67 ± 0.12	0.29

[a] NAMP# 115D or Deep Pectoral
[b] NO = No Mechanical Tenderization
[c] MT = Mechanical Tenderization
[d] Initial Juiciness:1=Extremely Dry, 8=Extremely Juicy
[e] Sustained Juiciness:1=Extremely Dry, 8=Extremely Juicy
[f] Initial Tenderness:1=Extremely Tough, 8=Extremely Over Tender
[g] Sustained Tenderness:1=Extremely Tough, 8=Extremely Over Tender
[h] Flavor Intensity:1=Extremely Bland, 8=Extremely Intense
[i] Off Flavor:1=Extreme Off Flavor, 4 = None
[j] Overall Acceptability:1=Dislike Extremely, 8=Like Extremely
Means with corresponding values ($P < 0.05$) differ.

Table 9. LS Means ± SE of Thiobarbituric Acid Analysis (TBA) for Treatment and Tenderization Method on Pectoral (n=119) and FLAT Muscles (n=120)

Enzymatic Treatment[a]	**TBA FLAT**[b]	**TBA PEC**[c]
BBA	0.07 ± 0.01[y]	0.06 ± 0.01
BF	0.13 ± 0.01[z]	0.04 ± 0.01
BP	0.08 ± 0.01[y]	0.06 ± 0.01
C	0.09 ± 0.01[y]	0.07 ± 0.01
FP	0.09 ± 0.01[y]	0.07 ± 0.01
PBA	0.08 ± 0.01[y]	0.04 ± 0.01
Tenderization Method[d]		
NO	0.08 ± 0.01	0.05 ± 0.01
MT	0.10 ± 0.01	0.05 ± 0.01

[a] Enzymatic Treatments: BBA = Bromelain and *Bacillus*, BF = Bromelain and Ficin, BP = Bromelain and Papain, C = Control, PBA = Papain and *Bacillus*, and FP = Ficin and Papain

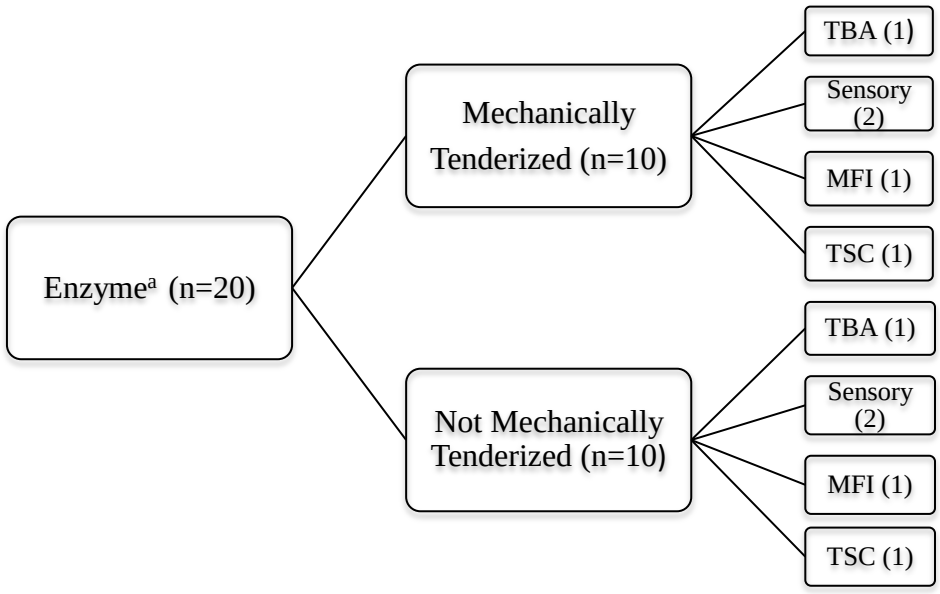
[b] NAMP# 171BL06 or *biceps femoris* (Treatment: P = 0.04; Tenderization: P = 0.13; and Treatment x Tenderization: P = 0.35)

[c] NAMP# 115D or Deep Pectoral (Treatment: P = 0.12; Tenderization: P = 0.99; and Treatment x Tenderization: P = 0.89)

[d] NO = No Mechanical Tenderization or MT = Mechanical Tenderization

[yz] Means within a muscle attribute combinations that lack common superscripts differ ($P \leq 0.05$)

Figure 1. Diagram of Treatment Assignment and Laboratory Analysis



[a]Enzyme combinations (ppm): BF = Bromelain (7.5) and Ficin (4.5), PBa = Papain (4.5) and *Bacillus* (4.4), BP = Bromelain (7.5) and Papain (4.5), FP = Ficin (4.5) and Papain (4.5), BBa = Bromelain (7.5) and *Bacillus* (4.4), or C = Control

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APPENDICES

APPENDIX A

MYOFIBRIL FRAGMENTATION INDEX (MFI) ASSAY

Myofibril Fragmentation Index (MFI) Assay

Reagents:

1. **MFI Buffer (2 LITERS), pH 7.0:** 250 ml MFI buffer per sample.

   100 mM KCl, 20 mM potassium phosphate, 1 mM EGTA, 1 mM MgCl2, 1 mM NaN3

KCL	14.91 g
KH2PO4	2.72 g
K2HPO4	3.50 g
EGTA	0.76 g
MgCl2	0.41 g
NaN3	0.13 g

   Dissolve in distilled deionized water. Adjust pH to 7.0. Bring to a final volume of 2 liters. Store at 4°C. Do not use anhydrous magnesium chloride, as this chemical causes a yellow tint.

2. **Biuret Reagent:** 16 ml per sample.

   Dissolve 1.5 g Cupric Sulfate ($CuSO_4 \cdot 5H_2O$) and 6 g sodium potassium tartrate (Rochelle Salt, $NaKC_4H_4O_6 \cdot 4H_2O$) in about 500 ml distilled deionized water in a 1000 ml volumetric flask. With constant stirring, add 300 ml of freshly prepared, carbonate free 10% NaOH. Bring up to 1 liter with distilled deionized water and store in a brown polyethylene bottle. Store at room temperature. Discard if a black or red precipitate appears.

Procedure:

Extraction

1. Sample extraction should be done in duplicate.
2. In a cold room (2°C), scissor mince 4 grams of muscle. Minced sample should be free of fat and connective tissue.
3. Put sample in a Eberbach blender container (Eberbach Semi-micro 350 ml stainless steel container with pressure fit lid, A. Daigger #LC22337A) and add 40 ml cold (2°C) MFI buffer. Using a blender (Waring commercial, 2 speed blender, A. Daigger #LC22302A), homogenize on high (22,000 rpm) for 30 seconds.
4. Pour the homogenate (with the aid of a funnel) into a 50 ml conical bottom centrifuge tube.
5. Centrifuge at 1,000 x g for 15 minutes (2°C).
6. Discard the supernatant. If there is a fat cap (layer of fat, connective tissue, and myofibrils) above the supernatant, save the fat cap with the pellet.
7. Using a glass stir rod, resuspend the pellet (and fat cap) in 40 ml cold (2°C) MFI buffer. (DO NOT USE A VORTEX MIXER).
8. Centrifuge at 1,000 x g for 15 minutes (2°C).

9. Discard the supernatant and fat cap.
10. Resuspend the pellet in 10 ml cold (2°C) MFI buffer and mix well by using a Vortex Mixer.
11. To remove connective tissue, pour the sample through a polyethylene strainer. Rinse the centrifuge tube with an additional 10 ml cold (2°C) MFI buffer and pour through the polyethylene strainer. (A Tupperware© strainer, 2" diameter, 1"height, 1 mm pore size, works well. Place the strainer on a funnel that has been placed a conical centrifuge tube).

Protein Assay

1. Protein assay should be conducted in duplicate for each sample suspension.
2. Place 0.25 ml of each sample into 13x100 mm glass tubes.
3. Add 0.75 ml MFI buffer.
4. Add 4 ml Biuret reagent and mix on a vortex mixer.
5. Incubate for 30 minutes at room temperature and in the dark.
6. Simultaneously, Bovine Serum Albumin (BSA) standards should be run to establish a standard curve used in determining protein concentration. The following concentrations are preferred: 0 (blank), 2.5, 5.0, 7.5, and 10.0 mg/ml. To these 1 ml standards, add 4 ml Biuret reagent and incubate for 30 minutes. Standards should be run in duplicate.
7. Read the absorbance at 540 nm using a Bausch and Lomb Spectronic 20 Spectrophotometer© with a large slit with (20 nm). If the spectrophotometer is properly calibrated, the absorbance of the standards should be approximately 0, 0.15, 0.30, 0.45 and 0.60 for the 0, 2.5, 5.0, 7.5, and 10.0 mg/ml BSA standards, respectively.

8. Using the standard curve, calculate the protein concentration (mg/ml) of the samples.

MFI Measurement

1. MFI should be measured in duplicate for each sample suspension.
2. In a 13x100 mm glass tube, dilute an aliquot of the sample suspension to equal 0.5 mg/ml protein in 8 ml MFI buffer.
3. Cap tube and mix sample immediately before reading the absorbance (540 nm) on the Spectronic 20 spectrophotometer. Use MFI buffer for the blank.
4. Multiply the absorbance reading by 200 to obtain the Myofibril Fragmentation Index.

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Reference: Culler, R.D., F.C. Parrish, Jr., G.C. Smith, and H.R. Cross. 1978. Relationship of myofibril fragmentation index to certain chemical, physical, and sensory characteristics of bovine longissimus muscle. J. Food Sci. 43:1177.

APPENDIX B

SENSORY EVALUATION

Sensory Evaluation

1. Steaks should have an internal temperature of 2.5°C before cooking. It is common to thaw steaks before cooking at 2-5°C for 12 – 24 hours.
2. Take care and maintain sample identity throughout process.
3. Pre-heat sample holding containers and pans. Pans with separate suspended compartments can be utilized, with the addition of sand below to maintain temperature.
4. Internal temperature of each steak should be taken in the geometric center of the steak and recorded. Temperature should be in the range of 2-5°C.
5. Weigh each steak in grams before cooking and record.
6. Place steak on cooking surface and cook until a medium degree of doneness. The internal temperature of steaks should be approximately 71°C.
7. Weight and temperature of each steak should be recorded immediately after cooking utilizing same procedures as before cooking.
8. Cut all four sides of the steak in a fashion that produces a square rectangle out of the steak, while removing fat and connective tissue.
9. Cut the remaining portion of the steak into 1 cm^3 pieces. Take care that all samples are devoid of fat and connective tissue.
10. Place all pieces of sample in designated sample holding container and maintain identity.
11. Panel room should be prepared before cooking to facilitate efficient panel time and minimized period after cooking until panel evaluation.
12. Panel ser up and evaluations should be according to Cross et al., 1978.
13. Record all sensory data for analysis.

APPENDIX C

THIOBARBITURIC REACTIVE SUBSTANCE (TBA) ASSAY

Thiobarbituric Reactive Sustance (TBA) Assay

modified from:

Buege and Aust. 1978. Methods in Ensymol. 52.302, AP

Reagents:

1. TCA/TBA stock solution: 15% TCA (w/v) and 20mM TBA (MW 144.15) reagents in DW. **Dissolve 2.88g TBA in warm DDW first, add TCA (150g) and then add DW to the mark (1L).** One liter last 100 samples in duplicate.

2. BHA: Make 10% stock solution by dissolving in 90% ethanol. Make 500ml batches.

3. TEP standard: $1 * 10^{-3}$ 1, 1, 3, 3-tetra-ethoxypropane in DW. This solution can be kept for about a week if stored in the refrigerator and diluted as needed. (MW 220.31, 95% purity, d = 0.981). Dilute 0.5 ml TEP with 499.5 ml DW, and dilute the resulting solution 1: 2.96 (TEP solution: DW) with DW.

Procedure:

1. Slice 10 g of fresh frozen meat and place in blender cup with 30 ml of DW.
2. Homogenize with a blender for 2 min. (or homogenize for 10-15 sec using a polytron at a speed 7-8.)
3. Take 2 ml of the homogenate, combine with 4 ml of the TCA/TBA reagent, 100 µl BHA, vortex thoroughly.
4. Heat the solution for 15 min in boiling water.
5. Cool for 10 min in cold water.
6. Vortex thoroughly.
7. Centrifuge at 2000G (3000RPM for 10 min).
8. Read the absorbance of the supernatant at 531 nm against a blank that contains all the reagents minus sample.

Malonaldehyde standard curves (CHO-CH^2, MW 72.0)

1. Construct TBA standard curve using TEP.
2. Label tubes: six tubes – 0 and two tubes of each – 5, 10, 20, 30, 40, 50
3. Add the following amount to each tube:

	TEP	DW	Set Pipettor on:
0	0 µL	2000 µL	1000 (twice)
5	10 µL	1990 µL	995 (twice)
10	20 µL	1980 µL	990 (twice)
20	40 µL	1960 µL	980 (twice)
30	60 µL	1940 µL	970 (twice)
40	80 µL	1920 µL	960 (twice)
50	100 µL	1900 µL	950 (twice)

4. Add 4 ml TBA/TCA to each tube, vortex.
5. Heat the tubes in boiling water bath for 15 min.
6. Cool in cool water bath for 20 min.
7. Vortex.
8. Read the optical density of the standard against a blank at the same wavelength (531 nm).